IN THE SPECIFICATION

Please replace paragraphs 5 through 7 of the disclosure with the following paragraphs 5 through 7:

[0005] Most detection assays on the market use a labelled substrate analogue. The detection of protease activity can be performed using a homogenous or a heterogenous reaction set up. In a homogenous detection assay, the substrate is typically in solution and the product is also in solution. Fluorescence-based detection systems using fluorophores (for example fluoroscein, rhodamine or BODIPY fluorophores) generally operate according to one of two principles: detection of a fluorescent signal following cleavage of a multiply labelled self-quenched protein (~~enzChek~~ ENZCHEK protease assay kits, Molecular Probes Inc.), or detection of a change in the size of the fluorescent-labelled moiety/conjugate using fluorescence polarization techniques (~~Beacon(R),~~ BEACON protease activity detection kit, PanVera Corporation, ~~enzChek~~ ENZCHEK polarization kit, Molecular Probes Inc.).

[0006] In one commonly practised method, a peptide substrate is used that is labelled at the carboxyl terminus with a dye possessing an amine functionality. Such a dye may be a chromophore or a fluorophore, for example coumarin, fluoroscein, rhodamine or BODIPY (Molecular Probes Inc, ~~apoalert(TM)~~ APOALERT, CPP32 protease assay kits, Clontech). The amide bond that couples dye and amino acid is cleaved by the protease to produce an amine derivative. That change in structure affects the spectral characteristics of the dye and a detectable signal is thus produced. An alternative strategy for homogenous detection is to use a peptide substrate that is labelled at one side of a cleavage site with a donor fluorophore and at the other side with an acceptor quencher, which together form a fluorescence resonance energy transfer (FRET) pair. Cleavage of the peptide results in separation of the donor and acceptor and therefore produces a change in fluorescence signal. Hydrolysis of a specific peptide sequence can be detected by a gel based analysis (~~Peptag~~ PEPTAG protease assay, Promega).

[0007] A heterogenous assay typically involves the cleavage of a dye-labelled fragment of an immobilised substrate and subsequent analysis of the liquid phase (~~ProteaseSpots~~ PROTEASESPOTS, Jerini AG, ~~ProCheck(TM)~~ PROCHECK Universal Protease Assay, Intergen).

Please replace paragraph 30 of the disclosure with the following paragraph 30:

[0030] The use of short peptides is preferred under certain circumstances because it allows the synthesis of substrates containing only the recognition sequences specific for the specific protease or class of proteases under investigation. For example, a substrate incorporating a short peptide of the sequence Ala-Ala-Phe or Ala-Ala-Pro-Phe (SEQ ID NO: 1), and preferably very little or no further sequence, may be used in a specific assay for chymotrypsin activity. As a further example, Leucine aminopeptidase (LAP) is a proteolytic enzyme that is an exopeptidase that hydrolyses peptide bonds adjacent to a free amino group. It shows specificity for peptide bonds next to leucine residues. Elevated serum and urine levels of LAP are seen in several clinical conditions including cholestasis, hepatic cirrhosis, hepatic necrosis, hepatic tumor, breast cancer, endometrial cancer, ovarian cancer, systemic lupus erythematosus and germ cell tumors of the ovary and testis. A substrate comprising a short peptide containing N-terminal alanine residues or a substrate comprising a labelled alanine amino acid, for example the substrate shown in formula IIIa, may be used to screen serum or urine samples for elevate levels of LAP in order to assist diagnosis of one of the above-listed conditions.

Please replace paragraph 33 of the disclosure with the following paragraph 33:

[0033] In many circumstances, however, it is not essential for a full length protein to be used. According to one embodiment of the invention the peptide preferably comprises at least 5, more preferably at least 20 amino acid residues. For example, the peptide may comprise from 20 to 100 amino acid residues; most preferably the peptide comprises from 20 to 50 amino acid residues. According to another embodiment of the invention, the substrate preferably comprises a single amino acid molecule. According to yet another embodiment of the invention, the substrate comprises a peptide from having little or nothing more than the protease recognition sequence. Such recognition sequences are often 2 to 6 amino acid residues in length, more preferably 3 amino acids in length. In practice, the length of the peptide is so selected that there is present at least one cleavage site for the enzyme or enzymes of interest. Preferably the peptide has one different cleavage site for the enzyme or enzymes of interest. For example, Factor Xa protease requires the presence of the recognition sequence Ile-Glu-Gly-Arg (SEQ ID NO: 2) in its substrate. Peptides having more than one cleavage site may be of use, for example, where the substrate is to be used in a screen for general protease activity.

Please replace paragraph 97 of the disclosure with the following paragraph 97:

[0097] NAP 10 columns (G25 DNA grade ~~sephadex~~ SEPHADEX) were obtained from Amersham Biosciences. ~~Trizma~~ TRIZMA hydrochloride (99+%), ~~Trizma~~ TRIZMA base (99+%), sodium chloride (~~SigmaUltra~~ SIGMAULTRA min. 99.5%), sodium acetate (molecular biology grade), ethylenediaminetetraacetic acid tetrasodium salt (~~SigmaUltra~~ SIGMAULTRA min. 99.0%), sodium hydroxide (~~SigmaUltra~~ SIGMAULTRA min. 98%), DL-cystein hydrochloride (min. 98%), hydrochloric acid, and molecular biology grade water were obtained from Sigma. ~~Ponceau~~ PONCEAU S (practical grade) ammonium persulfate (electrophoresis reagent), N,N,N',N'-tetramethyleneethylenediamine (TEMED),

acrylamide/bis-acrylamide (37.5:1), 30% solution and EZ Blue gel staining reagent were obtained from Sigma. Acetic acid (glacial, 99.99+%) was obtained from Aldrich and isopropanol was obtained from Hayman. ~~Biodyne~~ BIODYNE C membrane was obtained from Pall Life Sciences.

Please replace paragraph 99 of the disclosure with the following paragraph 99:

[0099] All solutions were prepared with autoclaved deionised water (~~WaterPro~~ WATERPRO system, Labconco). Materials and Methods-Electrochemical Detection

Please replace paragraph 105 of the disclosure with the following paragraph 105:

[0105] ~~AutoLab~~ AUTOLAB electrochemical workstation (either PGSTAT30 with frequency response analyser or ~~μAutoLab~~ μAUTOLAB type II) obtained from Windsor Scientific, Slough, Berkshire.

Please replace paragraph 107 of the disclosure with the following paragraph 107:

[0107] The low volume cell of FIG. 1 was filled with approximately 10 ml sodium chloride solution (100 mM). A 200 μl aliquot of the sample for analysis was placed in the glass sample chamber which was then placed in the low volume cell along with the reference and counter electrodes. The electrodes were connected to the ~~Autolab~~ AUTOLAB electrochemical workstation and differential pulse voltammetry carried out using the parameters described in Table 1. Prior to analysis the glassy carbon working electrode was polished (using BAS polishing kit catalogue number MF-2060) followed by conditioning. Electrode conditioning consists of cyclic voltammetry, sweeping between +-1 volt in the appropriate background buffer.

Please replace paragraph 119 of the disclosure with the following paragraph 119:

[0119] BSA concentration was determined by blotting onto ~~Biodyne~~ BIODYNE C membrane using BSA standard concentrations and staining with ~~Ponceau~~ PONCEAU S. Using the method, BSA concentrations were found to be 0.3-0.6 $mgml^{-1}$. Presence of the ferrocene label was confirmed by voltammetric analysis.

Please replace paragraph 126 of the disclosure with the following paragraph 126:

[0126] Baseline corrected data is displayed as overlayed files in addition to the raw data. Baseline corrected data was obtained using GPES ~~Manager~~ MANAGER (Ecochemie BV, Utrecht, Netherlands) selecting baseline correction from the edit data menu, selecting moving

Please replace paragraph 150 of the disclosure with the following paragraph 150:

[0150] Aminopeptidase was obtained as 20 Uml^{-1} in ammonium sulphate solution. The ferrocenylated alanine substrate of Example 4b was dissolved in ethanol at a 100 mM concentration. This was then diluted down to a working solution of 1 mM in 100 mM tris-HCl (pH 7.5). For a 200 µl volume, 195 µl of substrate solution (1 mM in tris-HCl/1% v/v ethanol) and 5 µl (0.01 U) of aminopeptidase were incubated for up to 15 minutes at 37° C. The sample was analysed by differential pulse voltammetry (DPV) before incubation, after 5 minutes incubation and after 15 minutes incubation. DPV was carried out as described in Example 1. Results are presented in FIG. 19a and FIG. 19b. FIG. 19a shows raw data and FIG. 19b shows the same data corrected for baseline using GPES ~~Manager~~ MANAGER as explained in Example 5. Line A shows the voltammetry trace for ferrocenylated alanine before incubation with amino peptidase, line B shows the voltammetry trace after 5 minutes

incubation with amino peptidase and line C shows the voltammetry trace after 15 minutes incubation with amino peptidase. It can be seen that digestion of the substrate causes the current peak potential to shift. After 15 minutes digestion, the shift is approximately 80 mV.

Please replace paragraph 151 of the disclosure with the following paragraph 151:

[0151] Elastase was obtained lyophilised and rehydrated in a 100 mM tris HCl buffer (pH 8.5) to 10 mg/ml. A 200 µl aliquot of test sample was prepared to contain 50 µl ferrocene labelled tripeptide substrate (ferrocenylated trialanine peptide prepared as in Example 4c), 1 µl elastase in tris HCl and the volume made up to 20011 with further addition of tris HCl buffer. The solution was incubated for 1 hour at 37° C. The sample was analysed by differential pulse voltammetry (DPV) before incubation and after 1 hour of incubation. DPV was carried out as described in Example 1. Results are presented in FIG. 20a and FIG. 20b. FIG. 20a shows raw data and FIG. 20b shows the same data corrected for baseline using GPES ~~Manager~~ MANAGER as explained in Example 5. Line A shows the voltammetry trace for the substrate before incubation with elastase, and line B shows the voltammetry trace after 1 hour incubation with elastase. It can be seen that digestion of the substrate causes the current peak potential to shift. However, the shift is smaller than that shown in Example 10.

Please enter the following page comprising SEQ ID NOs 1 and 2 of the "Sequence Listing".

<110> BRAVEN, Helen; KEAY, Russell; FLOWER, Stephen

<120> PROTEASE DETECTION ASSAY

<130> ATLAS 9452 US

<140> US 10/562,290
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<400> 2

Ile-Glu-Gly-Arg
1